APPLICATION NOTE

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Low level quantification of NDMA and non-targeted contaminants screening in drinking water using GC Orbitrap mass spectrometry

Authors: Cristian Cojocariu,[1] Maria José Farré,[2] Sara Insa,[2] Damià Barceló[2,3] and Paul Silcock[1]

[1]Thermo Fisher Scientific, Runcorn, UK; [2]Catalan Institute for Water Research (ICRA), Parc Científic i Tecnològic de la Universitat de Girona, 17003 Girona, Spain; [3]Institute of Environmental Assessment and Water Research, (IDAEA-CSIC) Water and Soil Quality Research Group, Department of Environmental Chemistry, 08034 Barcelona, Spain

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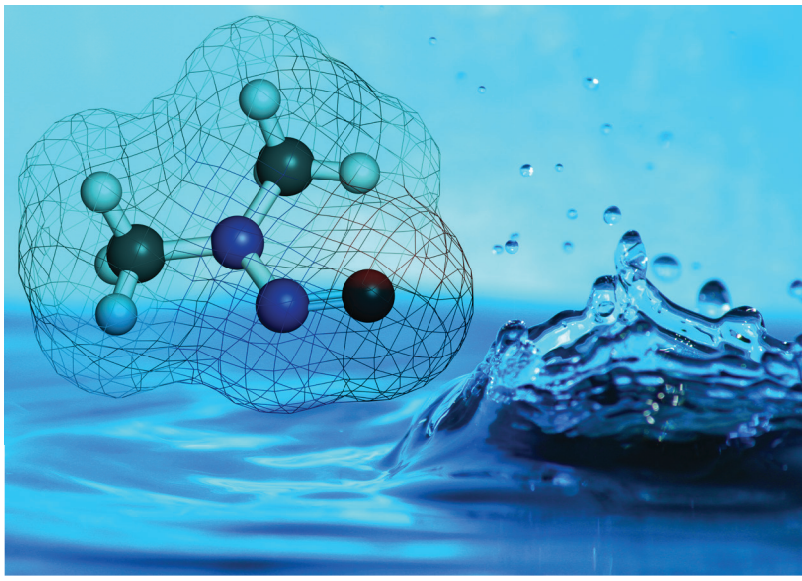
Introduction

N-nitrosodimethylamine (NDMA) is a semivolatile organic compound that belongs to nitrosamines, an emerging class of drinking water contaminants. NDMA is the main nitrosamine of concern and is classified as a potent carcinogen by the U.S. Environmental Protection Agency as it is known to induce tumors following administration by either ingestion or inhalation.[1] NDMA is formed as a by-product during industrial processes such as chloramination of wastewater and drinking water.[2] NDMA is considered a priority pollutant and various countries around the world have already introduced maximum acceptable concentrations of 9 ng/L[6] and action levels 10 ng/L.[7] It is particularly important that NDMA is detected and accurately quantified in drinking water as even low level of this chemical (e.g., 10 ng/L) can pose human cancer risks, being especially toxic to the liver.[1]



Traditionally, the analytical methodology used for NDMA detection and quantification employs single or triple quadrupole gas chromatography mass spectrometry (GC-MS), or magnetic sectors and high resolution time-of-flight mass spectrometers. However, with these analytical instruments it is difficult to obtain high selectivity and high sensitivity at the same time. Reduced selectivity can lead to interferences with the matrix and background chemical ions and can result in false positive detection and erroneous quantification of NDMA.[8] This is due to poor selectivity through insufficient resolving power of such instrumentation.

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In this work, a sensitive and selective method for NDMA detection and quantification using high resolution accurate mass GC Orbitrap technology is described. Test samples were subjected to GC-MS analysis using a Thermo Scientific™ Exactive™ GC Orbitrap mass spectrometer and the quantitative performance of this novel analytical platform was evaluated for sensitivity, mass accuracy, repeatability and linearity of response. In addition to targeted quantification of NDMA, acquiring the data using full-scan high resolution mode allowed for additional contaminants screening and identification in the drinking water samples without the need for separate sample injections or complicated experimental setup.

Experimental conditions

In the experiments described below, an Exactive GC Orbitrap mass spectrometer was used. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was obtained with a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD TG-1701MS, 30m × 0.25 mm × 0.25 µm film capillary column (P/N: 26090-1420). Additional details of instrument parameters are shown in Table 1 and Table 2.

Table 1. GC and injector conditions.

TRACE 1310 GC System Parameters	
Injection Volume (µL):	2.0
Liner:	Single gooseneck (P/N 4530924-UI)
Inlet (°C):	220
Inlet Module and Mode:	Split/Splitless: Surged Splitless
Surge Pressure (kPa):	385
Surge Duration (min):	1.0
Split Flow (mL/min):	80
Column Flow (mL/min)	1.5
Oven Temperature Program:	
Temperature 1 (°C):	35
Hold Time (min):	1
Temperature 2 (°C):	130
Rate (°C/min):	25
Temperature 3 (°C):	230
Rate (°C/min):	125
Hold Time (min):	6

Automated optimization of ion detection and mass calibration was done using perfluorotributylamine (PFTBA) to achieve mass accuracy of <0.5 ppm in <5 minutes. To ensure sufficient selectivity, data was acquired using 60,000 resolving power measured at Full Width at Half Maxima (FWHM) and at *m/z* 200 (Table 2). This is particularly important when NDMA detection is in matrices that contain a high chemical background that can potentially interfere with NDMA ions. These GC-MS settings ensured that chromatographic data was acquired with a minimum of 12 points/peak for consistent peak integration.

Table 2. Mass spectrometer conditions.

Exactive GC System Parameters	
Transfer Line (°C):	260
Ionization Type:	EI
Ion Source (°C):	230
Electron Energy (eV):	70
Acquisition Mode:	Full-scan
Mass Range (*m/z*):	50–650
Mass Resolution (FWHM at *m/z* 200):	60,000
Lockmass (*m/z*):	207.03235

Samples

NDMA analysis involves a solid phase extraction (SPE) of the water samples that concentrate the extracts by a factor of 1000.[3] Taking this into account, the quantification performance of the Exactive GC-MS was tested using both solvent standards and real drinking water (tap water) samples.

The solvent standards were prepared in dichloromethane (DCM) and were spiked with native NDMA in DCM in a similar manner as for real water samples. The final concentration levels in the standards were: 0.1, 1, 10 and 100 µg/L (ppb). Each solvent standard was spiked with 20 µg/L deuterated NDMA (d_6-NDMA) in DCM which was used as an internal standard. In addition to these calibration standards, a procedural blank (DCM not spiked) was used (Table 3).

To validate the results from the solvent standard experiment, three drinking water samples (M1, M5 and M10) were collected in duplicate from the local ICRA facility and spiked with native NDMA prior to SPE extraction at three concentration levels: 0.96, 4.8 and 9.6 ng/L. In addition to these, a drinking water sample that was not spiked with NDMA (M0) was used as matrix blank. Each water sample was subjected to individual SPE extraction (EPA 521/522, Restek) followed by a concentration step

Table 3. Sample preparation for two separate experiments: top table details the preparation of solvent standards used for testing linearity, sensitivity, peak area repeatability; bottom table shows the solvent standards and drinking water samples used to validate the method for NDMA quantification.

Calibration standard	Working solution NDMA (µg/L)	Volume	NDMA conc. (µg/L)	d_6-NDMA 5 ppm (mg/L) added prior to adjusting the volume in the 10 mL flask	Final conc. (µg/L) d_6-NDMA
Cal 6	5000	200 µl in 10 mL flask (DCM)	100	40 µl	20
Cal 5	5000	20 µl in 10 mL flask (DCM)	10	40 µl	20
Cal 4	5	2000 µl in 10 mL flask (DCM)	1	40 µl	20
Cal 3	5	200 µl in 10 mL flask (DCM)	0.1	40 µl	20
Cal 1	0	Blank no NDMA added (DCM)	0	40 µl	20

	NDMA concentration (µg/L)	d_6-NDMA (µg/L)	d_{14}-NDPA (µg/L)
Solvent standards in DCM	**0.0**	**0.0**	**24.0**
Cal 0	0.0	0.0	24.0
Cal 0.1	0.1	0.1	24.0
Cal 1	1.0	1.1	24.0
Cal 2	1.9	2.2	24.0
Cal 10	9.6	10.9	24.0
Cal 20	19.3	21.7	24.0
Cal 50	48.1	54.3	24.0
Tap water samples	**NDMA spiked prior to SPE (ng/L)**	**d_6-NDMA (µg/L)**	**d_{14}-NDPA (µg/L)**
M0A (blank tap water)	0.0	24.0	24.0
M0B (blank tap water)	0.0	24.0	24.0
M1A	1.0	24.0	24.0
M1B	1.0	24.0	24.0
M5A	4.8	24.0	24.0
M5B	4.8	24.0	24.0
M10A	9.6	24.0	24.0
M10B	9.6	24.0	24.0

to a 1.0 mL final volume in accordance to the EPA 521 method.[3] To correct for recovery, d_6-NDMA was spiked prior to SPE in each of the tap water samples at 24 ng/L level and used as a surrogate. Also, to correct for sample injection, d_{14}-N-nitrosodipropylamine (d_{14}-NDPA) was used as an internal standard and was added to the final 1.0 mL extract in each sample and standard at 24 pg/µL level.

Data processing

Data was acquired and processed using the Thermo Scientific™ TraceFinder™ software which allows for easy set-up and complete quantitative and qualitative analysis workflows. This includes peak integration, calculation of compound concentration and recoveries as well as easy data review and reporting. In addition, for qualitative analysis, TraceFinder automatically generates clean mass spectra following automated peak deconvolution and, compound identification (by searching a custom made, NIST compatible accurate mass library and commercially available spectral libraries).

Results and discussion

The objective of the analysis was to assess the use of GC Orbitrap technology for the analysis of NDMA in drinking water samples at a very low concentration level and for a broad scope non-targeted screening of the samples for the detection and identification of additional contaminants.

NDMA chromatography, sensitivity, linearity and peak area repeatability were evaluated using solvent based standards. This was followed by validation of the method using drinking water samples that were spiked with NDMA at low levels prior to SPE extraction and concentration. In addition to NDMA quantification, the water samples were also screened, using a non-targeted approach, for the presence of additional chemical contaminants. Putative identifications based on NIST library matches, fragment ion rationalization and accurate mass information were made.[4]

Chromatography and resolution

Using the GC conditions stated in the Table 1, fast GC separation (total GC run time 11 min), with good chromatographic separation was obtained, allowing for a high sample throughput. An example of chromatography for NDMA in the lowest calibration solvent standard (0.1 µg/L) and in the lowest level spiked drinking water sample (0.96 ng/L) is shown in Figure 1.

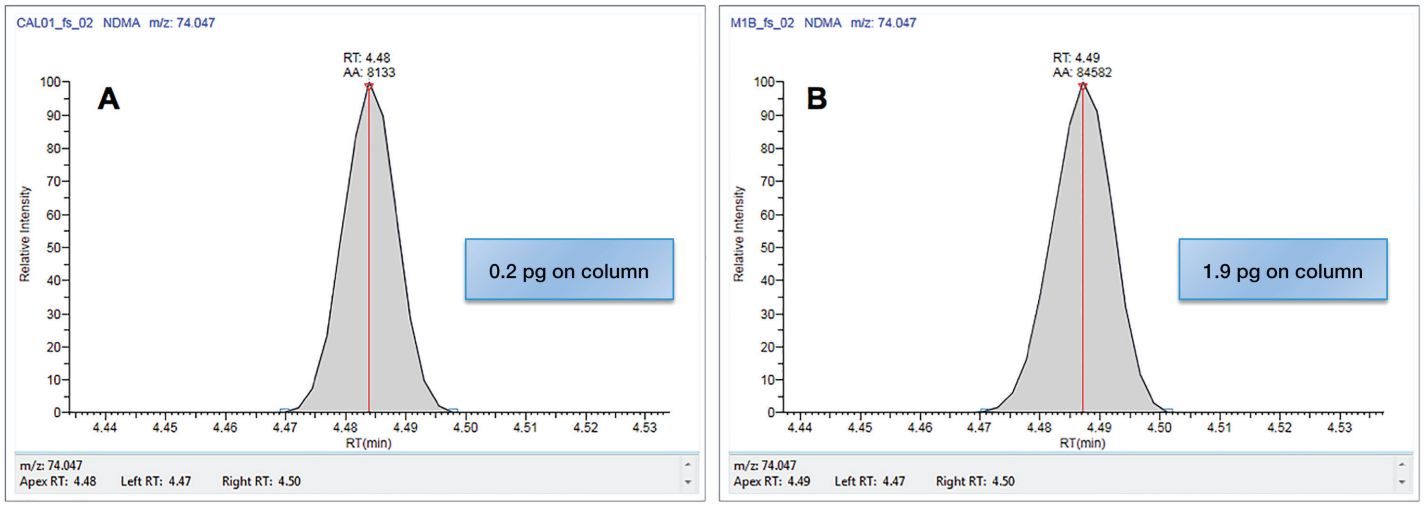
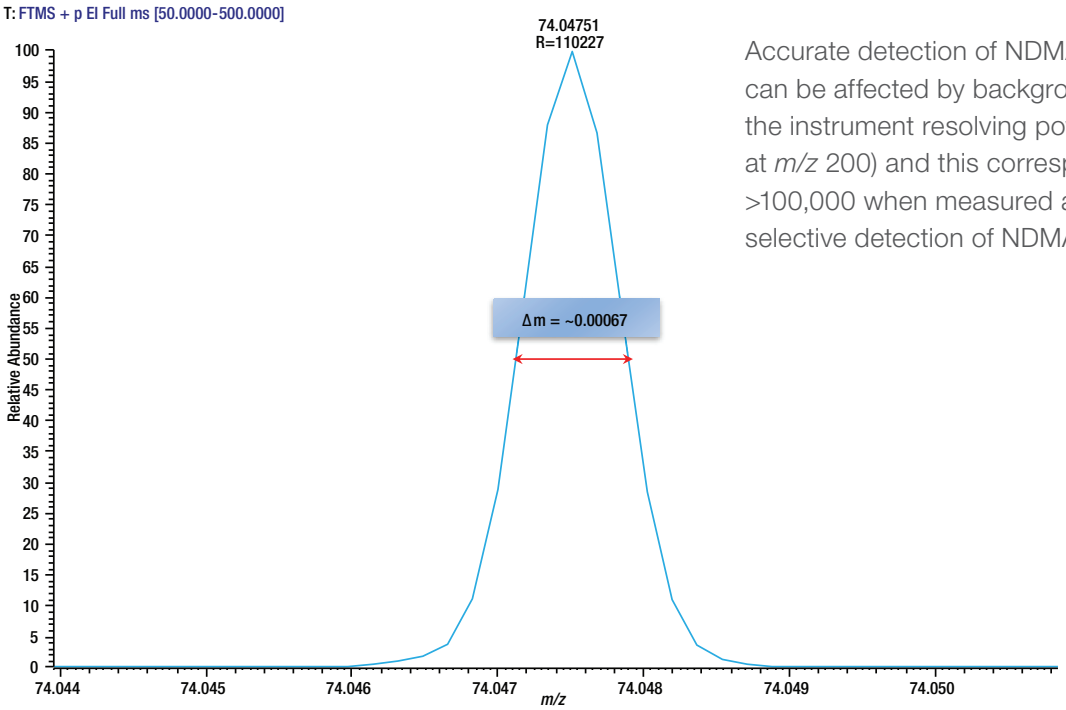


Figure 1. Extracted ion chromatogram (XIC, EI at 70eV) of the *m/z* 74.04747 corresponding to NDMA molecular ion at 0.1 µg/L in the lowest calibration solvent standard (A) and at 1.0 ng/L in a drinking water sample (B). The absolute amount on column is shown as pg of NDMA on column.



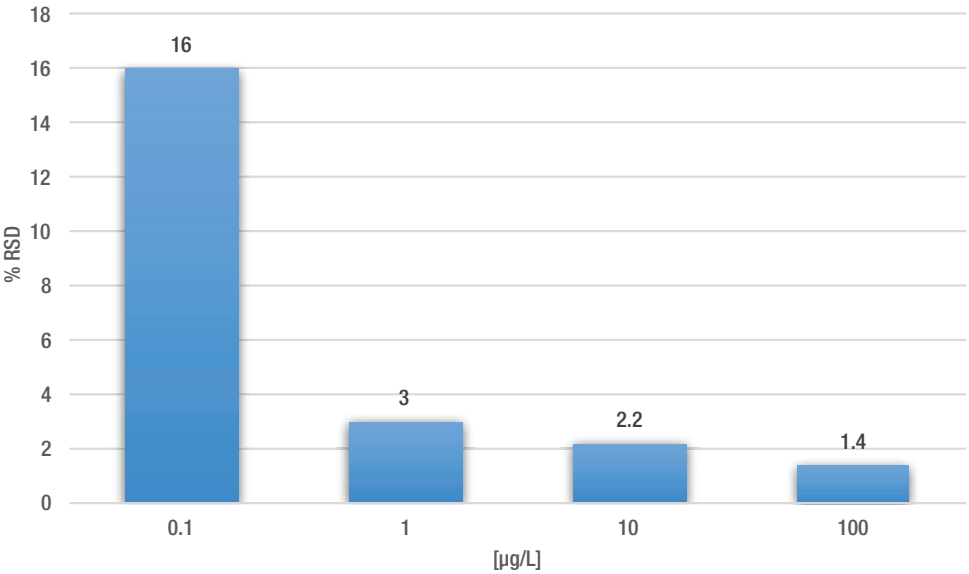
Accurate detection of NDMA molecular ion (*m/z* 74.04747) can be affected by background ions. In all experiments, the instrument resolving power was set to 60,000 (FWHM at *m/z* 200) and this corresponded to a mass resolution of >100,000 when measured at *m/z* 74, sufficient to achieve selective detection of NDMA target ions in matrix (Figure 2).

Figure 2. NDMA in a drinking water sample showing a mass resolution R> 110,000 (FWHM) measured at *m/z* 74.04747. Data acquired in full-scan using electron ionization at 70 eV.

Estimated Instrument Detection Limit (IDL) and peak area repeatability

System sensitivity was assessed by calculating the minimum quantifiable limit or the instrument detection limit (IDL) for NDMA. This was done by using the peak area %RSD derived from n=9 repeat injections of the lowest calibration standard 0.1 µg/L and taking into account the Student's-t critical values for the corresponding degrees of freedom (at 99% confidence). The results of this experiment showed that IDL derived from the Exactive GC system data was 0.09 µg/L, a value similar to the lowest calibration standard detectable.

For reliable quantification robust instrumental response is important, and this was demonstrated by assessing the peak area repeatability of NDMA quantification ion (*m/z* 74.04747). To achieve this, each solvent standard was injected five times except for the 1.0 µg/L standard which was injected nine times. Absolute peak area repeatability was evaluated by looking at %RSD at each concentration level and the results obtained are shown in Figure 3 below.



*n=5 injections per calibration standard were used except 1.0 µg/L level; where n=9 inj. were used

Figure 3. Absolute peak area repeatability of NDMA at various concentration levels (n=5 for 0.1, 10 and 100 µg/L and n=9 for 1.0 µg/L). The average %RSD values at each level are indicated above each bar.

Excellent peak area repeatability was demonstrated using the two batches of samples. For the experiment using solvent standards, across a total number of injections of n=35 the %RSD calculated from the peak area response of the d_6-NDMA internal standard was <4.5%, whereas the %RSD peak area response of the d_{14}-NDPA across n=14 injections (including water samples) was ~5% (Figure 4).

Mass accuracy

It is well known that analyte selectivity increases with higher mass accuracy, therefore obtaining consistent sub ppm accurate mass information provides distinct advantages in complex matrices and for the confident characterization and confirmation of a target chemical. In this study, the mass accuracy for NDMA *m/z* 74.04747 was always <1 ppm at low and high levels in both solvent standards and in extracted drinking water samples (Figure 5 and Table 4).

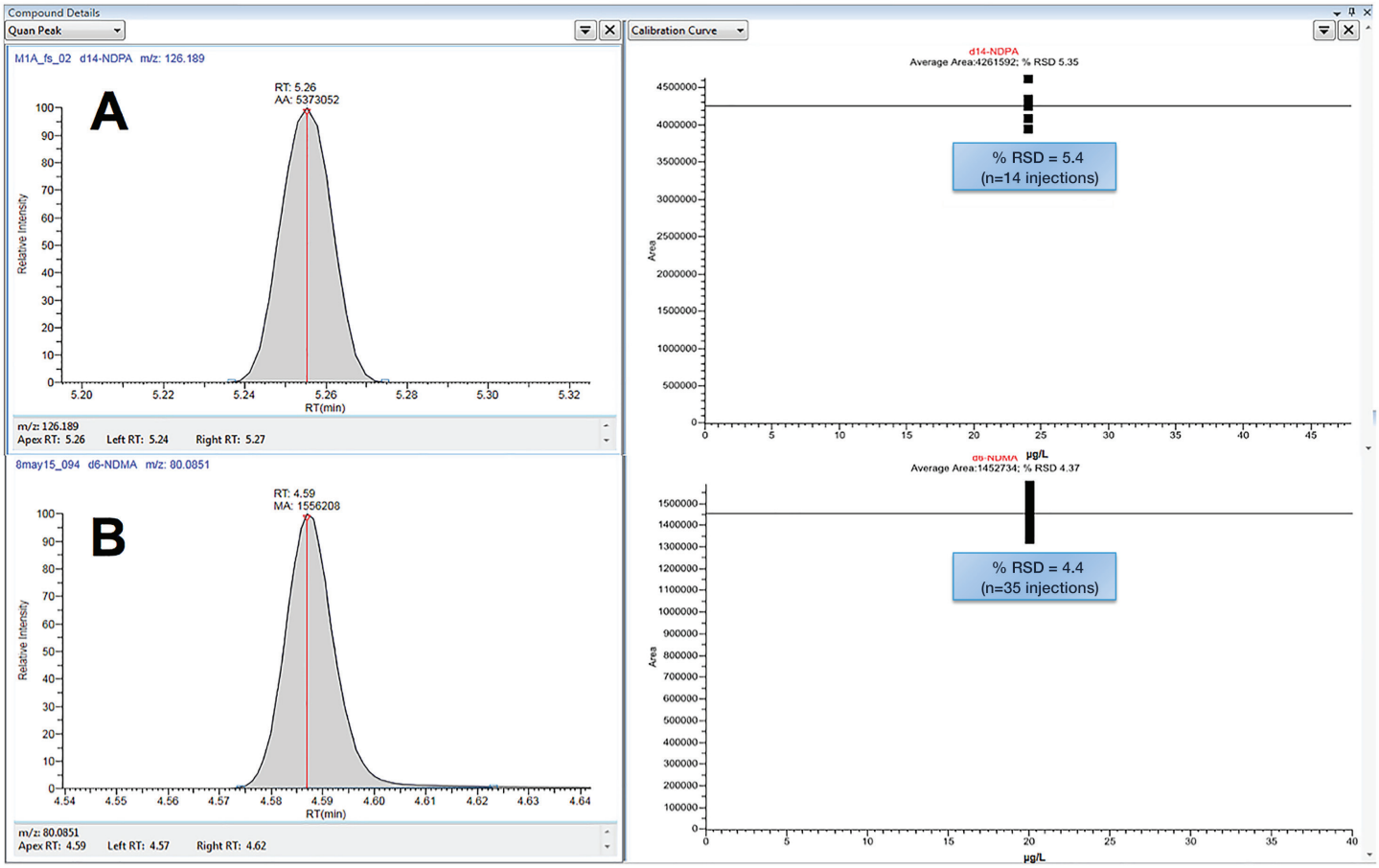


Figure 4. Peak area repeatability (as %RSD) demonstrated for two internal standards corresponding to two different experimental batches: d_{14}-NDPA internal standard across n= 14 injections (A), and d_6-NDMA internal standard across n= 35 injections (B).

Linearity of response

Quantitative linearity was assessed across a 7-point concentration range of 0.1, 1.0, 2.0, 10, 20 and 50 µg/L (ppb) including a solvent blank, each injected twice. Calibration linearity, assessed using a 1/x weighted linear regression, showed that the coefficient of determination (R^2) was >0.999 (Figure 6). Moreover, the %RSD of the relative response factors (RRF) for native NDMA was <9% and for its corresponding d_6-NDMA surrogate the %RSD RF was 6% (Figure 6).

Quantification of NDMA in drinking water samples

Calculated NDMA concentrations in the drinking water samples show good accuracy of the method (Table 4). Surrogate d_6-NDMA recovery was monitored throughout the entire sample sequence with the recovery values obtained in very good agreement with the method 521, which requires that surrogate recovery should be within 70–130% (Table 4). Overall, these results indicate that the Exactive GC mass spectrometer delivers excellent results and is highly suitable for routine laboratory use.

Table 4. Quantification results in drinking water samples

Sample	% recovery d_6-NDMA	Calculated NDMA concentration (ng/L)	Mass error [ppm]
M1A	107	1.1	0.7
M1B	105	0.96	0.01
M5A	111	4.7	0.01
M5B	104	4.3	0.01
M10a	88	8.4	0.2
M10b	99	8.1	0.1

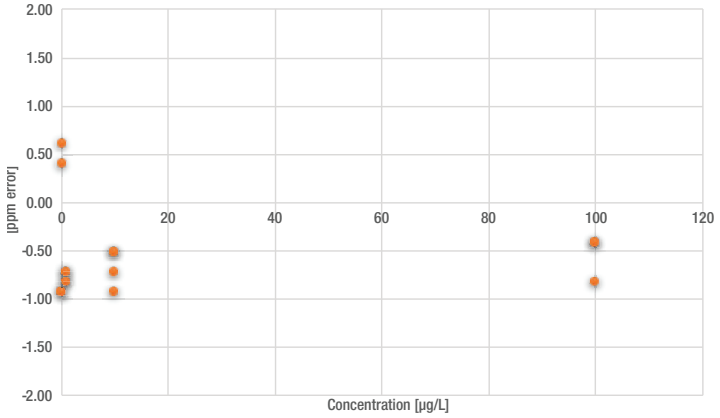


Figure 5. Mass accuracy measurements for NDMA quantitation ion *m/z* 74.04747. NDMA concentration levels (µg/L on the X-axis) as well as the corresponding mass error (ppm Y-axis) are shown. Each dot represents a separate injection.

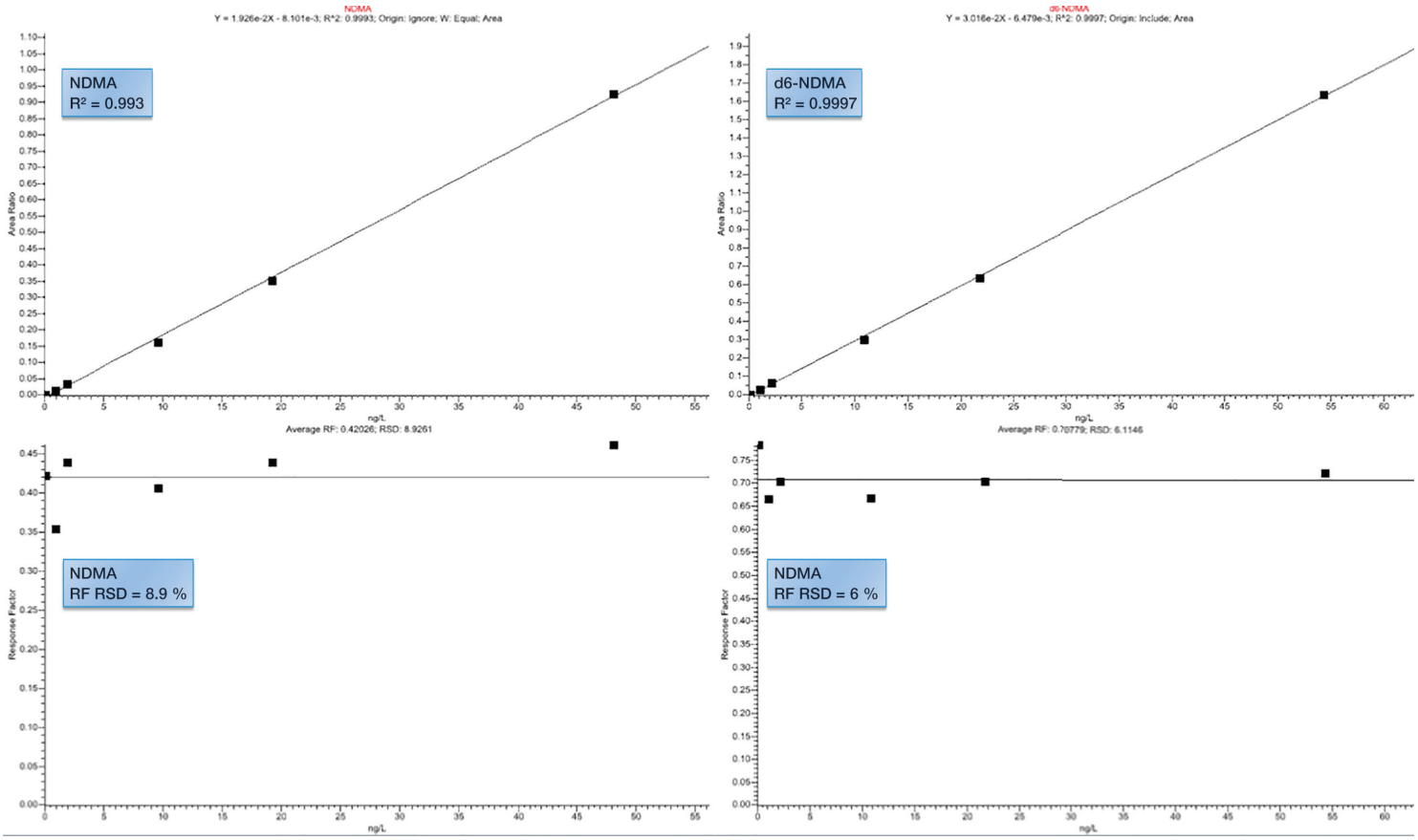


Figure 6. Linearity of NDMA (left) and d_6-NDMA (right) internal standard corrected with d_{14}-NDPA over a 7-point calibration curve (0.1-50 µg/L) showing the corresponding %RSD RF <9% for NDMA and 6% for the d_6-NDMA surrogate.

Non-targeted screening of drinking water samples for additional contaminants

A significant advantage of Exactive GC technology is that, due to its full-scan high resolution mode of operation, the analyst can screen the raw data used for the quantitation experiment for additional, potentially harmful chemical contaminants. This was demonstrated in this work using the data acquired from the drinking water samples which was subjected to a non-targeted screening workflow with TraceFinder. This workflow automates compound deconvolution to obtain clean ion spectra that is then submitted to a library search for putative compound identification. A detailed description of this workflow is described elsewhere.[4]

The results of this data processing show that the drinking water samples contain 220 additional chemicals not present in the procedural DCM solvent blank. These compounds were putatively identified using NIST library (using a forward search index threshold of 800) and a high resolution filtering score (HRF) threshold of 80. The HRF uses the accurate mass information to explain a NIST (or similar) library matched ion spectra.[4] The majority of the contaminants found in the drinking water samples are halogenated organic compounds, pharmaceuticals (ex: Clindamycin, Felbamate), monoterpenes (D-limonene) and phthalates etc. Examples of chemicals detected and identified with high confidence are shown in Figure 7. Chloroiodomethane has been previously reported in the literature as a disinfection by-product.[4] Also, tetrachloroethylene is a widely used as a dry cleaning chemical often found in private or public drinking water and it is known to adversely affect human health.[5] Both chemicals were identified with an excellent library match (SI >890), a total score >95% and a mass accuracy for the molecular ions <0.5 ppm.

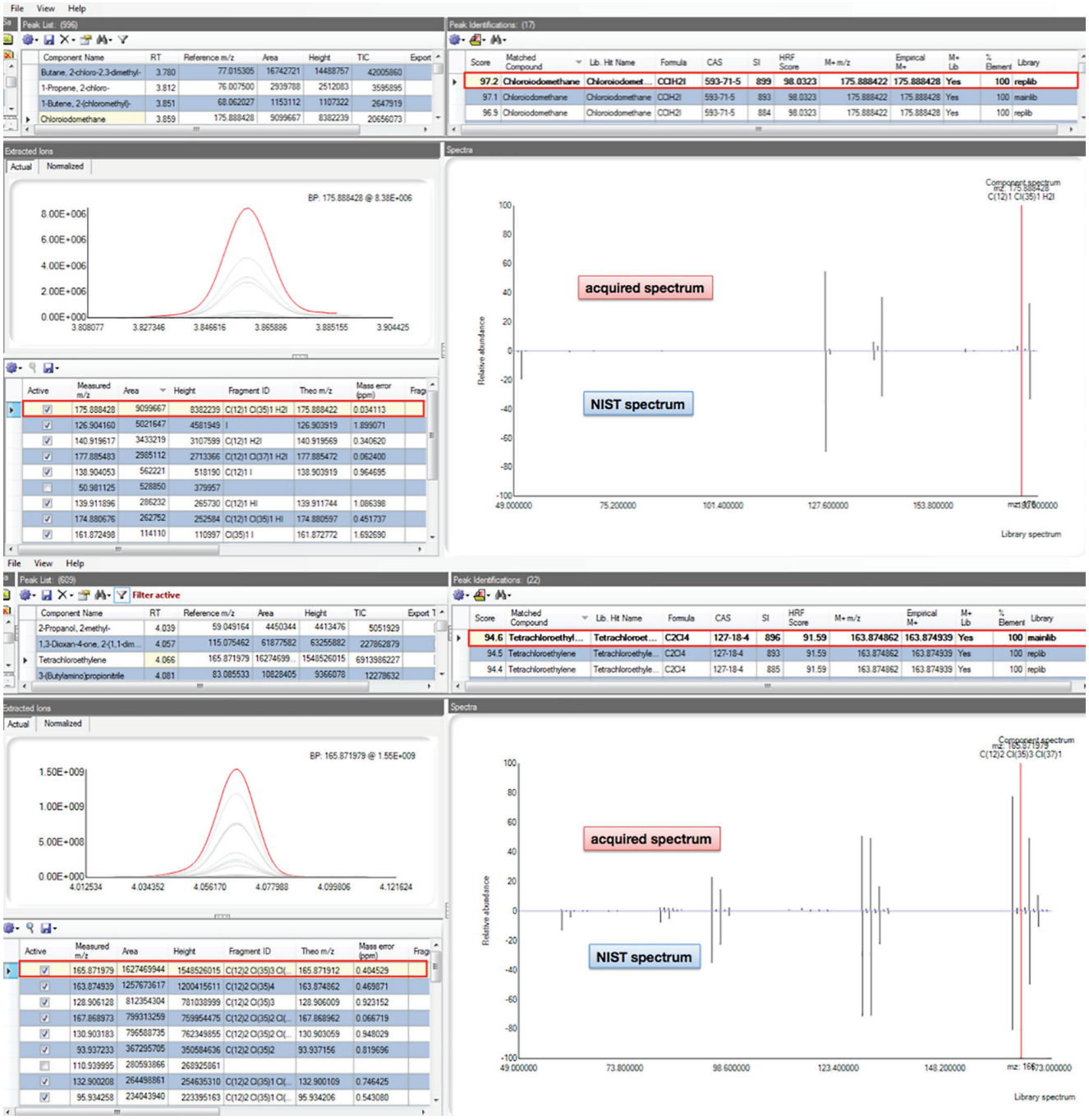


Figure 7. Examples of chemical contaminants present in the drinking water sample. TraceFinder deconvolution browser highlighting chloroiodomethane (a) and tetrachloroethylene (b) with their corresponding deconvoluted mass spectra, the total identification confidence score, NIST library match (forward SI) and accurate mass measurements for each of the measured ions.

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Conclusions

With the Exactive GC system in full-scan operation at 60,000 resolution (FWHM), NDMA was detected at 0.1 µg/L level in the lowest calibration level, which, assuming 100% recovery, will translate to an NDMA limit of detection (LOD) of 0.1 ng/L. In addition, NDMA was easily detected and accurately quantified at 1.0 ng/L in the drinking water samples with excellent recovery values.

Full-scan acquisition enabled the detection and putative identification of additional harmful contaminants in the drinking water samples. Halogenic organic compounds were predominantly detected and their presence is most probably related to the disinfection processes that involves chloramination and chlorination reactions. Putative identifications require further confirmation using analytical standards.

In addition to very high sensitivity, excellent linear response across 0.1–50 µg/L was observed for both NDMA (R^2 >0.999 and residuals <9 %RSD RF) and for its corresponding d^6-NDMA surrogate (R^2 >0.999 and residuals <6 %RSD RF).

Moreover, consistently low (sub ppm) mass deviation from the theoretical NDMA mass was observed at all concentration levels and in all analyzed samples.

Taken together, these results described in this study demonstrate excellent quantitative and qualitative performance of the Exactive GC system for the analysis of trace levels of NDMA.

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